

AN EVALUATION OF PHYSICO CHEMICAL, PHYTOCHEMICAL AND
BIOCHEMICAL ANALYSIS OF SIDDHA HERBAL FORMULATION
“MAAVILINGAPATTAI CHOORANAM

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ABSTRACT

The aim of the present study was to standardize the *Siddha* polyherbal formulation *Maavilingapattai Chooranam* which mentioned in the classical *Siddha* literature “*Sirorathina Vaidhiya Booshanam*” written by *Angamuthu Mudhaliyar*. Standardization of the drug (MPC) is mainly on the basis of organoleptic characters, physico chemical, phytochemical, biochemical and HPLC analysis. Standardization of the herbal drug is more important to assess its purity, quality, safety and efficacy of the drug. Thus the result of the study may give valuable information for further clinical studies.

KEYWORDS: *Maavilingapattai Chooranam*, MPC, Physicochemical, Phytochemical, Biochemical.

1. INTRODUCTION

Siddha system of medicine is one of the ancient system of medicine in India. The term *Siddha* is derived from the word ‘*Siddhi*’. *Siddhi* means attainment of perfection, accomplished or achievement. The unique nature of this system is its continuous service to humanity.^[1] The *Siddha* Herbal formulation “*Maavilingapattai Chooranam*” was taken as the compound drug preparation for Hepatoprotective activity mentioned in the classical *Siddha* literature “*Sirorathina Vaidhiya Booshanam*” written by *Angamuthu Mudhaliyar*, published by *Thamarai Noolagam*, Chennai-26, pg.no:148-149.

Nowadays the need for herbal medicines is increasing daily. So there is a demand to provide the quality of the drug. It is essential to standardize the herbal medicines for assess the safety and quality of the drug. Through this study, the organoleptic characters, physico chemical, phytochemical, biochemical and HPLC analysis of MPC may carried out which may give valuable information for future clinical studies.

2. MATERIALS AND METHODS

Drug selection

Table 1: Ingredients of *Maavilingapattai Chooranam*.

S.no	Name of drugs	Botanical name
1.	<i>Iruveli</i>	<i>Vetiveria zizanioides</i>
2.	<i>Vilamichu</i>	<i>Plectranthus vettiveroides</i>
3.	<i>Chiru kurinchan</i>	<i>Gymnema sylvestre</i>
4.	<i>Poonai vanangi</i>	<i>Acalypha indica</i>
5.	<i>Kozhunji</i>	<i>Tephrosia purpurea</i>
6.	<i>Koovilam</i>	<i>Aegle marmelos</i>
7.	<i>Pathiri</i>	<i>Stereospermum colais</i>
8.	<i>Thulasi</i>	<i>Ocimum sanctum</i>
9.	<i>Musumusukkai</i>	<i>Mukia maderaspatana</i>
10.	<i>Musuttai</i>	<i>Rivea ornate</i>
11.	<i>Vila</i>	<i>Limonia acidissima</i>
12.	<i>Nannari</i>	<i>Hemidesmus indicus</i>
13.	<i>Kurundhotti</i>	<i>Sida rhombifolia</i>
14.	<i>Ashwagandhi</i>	<i>Withania somnifera</i>
15.	<i>Parangichakkai</i>	<i>Smilax china</i>
16.	<i>Maavilingapattai</i>	<i>Crataeva magna</i>
17.	<i>Seenisarkarai</i>	<i>Saccharum officinarum</i>

Collection of the Plant materials

The required raw materials were procured from a well reputed indigenous drug shop from Chennai, Tamilnadu.

Identification and Authentication of the drug

The raw materials were identified and authenticated by the experts of *Gunapadam*, Government Siddha Medical College, Arumbakkam, Chennai- 106. The specimen sample of each raw material has been kept in the PG *Gunapadam* department individually for future reference.

Purification of the drugs

Purification process was done as per classical *Siddha* literature.^[2]

Preparation of the trial drug *Maavilingapattai Chooranam*

All the purified raw materials (*Iruveli*, *Vilamichu*, *Chirukurinchan*, *Poonaivanangi*, *Kozhunji*, *Koovilam*, *Pathiri*, *Thulasi*, *Musumusukkai*, *Musutai*, *Vila*, *Nannari*, *Kurundhotti*, *Ashwagandhi* - each 35grams, *Parangichakkai* -70grams, *Maavilingapattai* - 280grams) were taken and powdered separately. Then all the powder were mixed together. Finally, the mixture was ground well which favours the homogenous preparation. Then the mixture of the powder was sieved through the thin clean white cloth. After that one third of the chooranam weight of sugar was added to the mixture and again it was ground well.

Finally, the end product was obtained, which was kept in an air tight container and labeled as "*Maavilinga Pattai Chooranam*" (MPC).

Purification of the Chooranam- Steaming process (*Pittaviyal murai*)

The "*Maavilinga Pattai Chooranam*" was purified by *Pittaviyal* method (steam cooking in milk) as per *Siddha* classical literature.^[3]

Storage of the drug

The prepared test drug was stored in a clean, air tight glass container.

Administration of the drug:

- Form of the medicine : *Chooranam*
- Route of Administration : Internal
- Dose : 2 gm twice a day depending on the severity
- Duration : 12- 24 Days.

Indication

Kamalai, *Pun*, *Purai*, *Megavettai*, *Megapadai*, *Themal*.

Standardization of the drug

Standardization of the drug brings the validation to be used as a medicine by subjecting the drug to many analysis and determining its quality and effectiveness. Standardization includes many studies such as its organoleptic properties, physical characteristics and phytochemical properties and also to assess the active principles and elements present in the drug.

Organoleptic character^[4]

The organoleptic characters of the sample were evaluated which include evaluation of the formulation by its colour, odour, taste, texture etc.

Physicochemical analysis^[5]

Physicochemical studies of the trial drug have been done according to WHO guidelines. Physico-chemical studies like total ash, water soluble ash, acid Insoluble ash, water and alcohol soluble extract, loss on drying at 105°C and pH were done at, Dr. MGR University, Chennai.

1. Solubility Test

A pinch of sample (MPC) was taken in a dry test tube and to it 2 ml of the solvent was added and shaken well for about a minute and the results are observed. The test was done for solvents like distilled water, Ethanol, Petroleum ether, Propylene glycol, Toluene, Benzene, Chloroform, Ethyl alcohol, Xylene, Carbon tetra chloride and the results are observed individually.

2. pH value

Potentiometrically, pH value is determined by a glass electrode and a suitable pH meter. The pH of the *Maavilingapattai Chooranam* was written in results column.

3. Loss on Drying

An accurately weighed 2gm of *Maavilingapattai Chooranam* formulation was taken in a tarred glass bottle. The crude drug was heated 105⁰ c for 6 hours in an oven till a constant weight. The percentage moisture content of the sample was calculated with reference to the shade dried material.

4. Determination of total Ash

Weighed accurately 2g of *Maavilingapattai Chooranam* formulation was added in crucible at a temperature 600⁰c in a muffle furnace till carbon free ash was obtained. It was calculated with reference to the air dried drug.

5. Determination of acid insoluble ash

Ash above obtained was boiled 5min with 25ml of 1M hydrochloric acid and filtered using an ash less filter paper. Insoluble matter retained on filter paper was washed with hot water and filter paper was burnt to a constant weight in a muffle furnace. The percentage of acid insoluble ash was calculated with reference to the air dried drug.

6. Determination of water soluble ash:

Total Ash 1g was boiled for 5min with 25ml water and insoluble matter collected on an ash less filter paper was washed with water and ignited for 15 min at a temperature not exceeding 450⁰c in a muffle furnace. The amount of soluble ash is determined by drying the filtrate.

7. Determination of water soluble extractive

5gm of air dried drug. Coarsely powdered *Maavilingapattai Chooranam* was macerated with 100ml of distilled water in a closed flask for twenty-four hours, shaking frequently. The solution was filtered and 25 ml of filtered was evaporated in a tarred flat bottom shallow dish, further dried at 1000⁰c and weighed. The percentage of water soluble extractive was calculated with reference to the air dried drugs.

8. Determination of alcohol soluble extractive

2.5gm of air dried drugs coarsely powdered *Maavilingapattai Chooranam* was macerated with 50ml alcohol in closed flask for 24 hours with frequent shaking. It was filtered rapidly taking precaution against loss of alcohol. 10ml of filtrate was the evaporated in a tarred flat bottom shallow dish, dried at 1000⁰c and weighed. The percentage of alcohol soluble extractive was calculated with reference to air dried drug.

Phytochemical analysis

The Phytochemical screening of the extract gives general idea regarding the nature of chemical constituents present in the crude drug. The phytochemical tests were done as the method illustrated.^[6]

Test for Alkaloids

Extracts were dissolved in dilute hydrochloric acid and filtered.

- 1) **Mayers's test:** Filtrates were treated with Mayer's reagent (Potassium, Mercuric iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.
- 2) **Wagner's test:** Filtrates were treated with Wagner's reagent (Iodine in potassium iodide). Formation of brown/ reddish precipitate indicates the presence of the alkaloids.
- 3) **Dragendroff's test:** Filtrates were treated with Dragendroff's reagent (solution of Potassium with Bismuth iodide). Formation of red precipitate indicates the presence of alkaloids.
- 4) **Hager's test:** Filtrates were treated with Hager's reagent (saturated picric acid solution) presence of alkaloids conformed by the formation of yellow precipitate.

Test for Carbohydrates and Reducing Sugars

Extracts were dissolved individually in 5ml distilled water and filtered. The filtrates were used to test the presence of carbohydrates.

- 1) **Molisch's test:** To 2ml of a plant sample extract, two drops of alcoholic solution of alpha naphthol are added. The mixture is shaken well few drops of concentrated sulphuric acid slowly along the sides of the test tube. A violet ring indicates the presence of carbohydrates.
- 2) **Benedict's test:** Filtrates were treated with Benedict's reagent and heated gently orange red precipitate indicates the presence of reducing agents.

Test for Glycosides

Extracts were hydrolysed with dilute HCl and then subjected to the test of glycosides.

- 1) **Modified bortrager's test:** Extracts were treated with ferric chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose pink colour in ammonical layer indicates the presence of anthranol glycosides.
- 2) **Cardiac glycoside (keller-killiani test):** Extracts was shaken with distilled water (5ml). to this, glacial acetic acid (2ml) containing few drops of ferric chloride was added followed by sulphuric acid (1ml) along the side of the test tube. The formation of the brown ring at the interface gives positive indication for cardiac glycoside and a violet ring may appear below the brown ring.

Test for Saponins

- 1) **Froth test:** Extracts were diluted with distilled water to 20ml and this was shaken graduated cylinder for 15 minutes. Formation of 1cm layer of foam indicates the presence of saponins.
- 2) **Foam test:** 0.5gm of extract was shaken with 2ml of water if foam produced persists for ten minutes. It indicates the presences of saponins.

Detection of phytosterols

- 1) **Salkowski's test:** Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of conc. sulphuric acid shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

Detection of phenol ferric chloride test

Extracts were treated with 3- 4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of the phenols.

Detection of tannins Gelatin test

The extract is dissolved in 5ml distilled water and 2ml of 1% solution of Gelatin containing 10% NaCl is added to it. White precipitate indicates the presence of phenolic compounds.

Detection of flavonoids

- 1) **Alkaline Reagent Test:** Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.
- 2) **Lead acetate Test:** Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Detection of Proteins and Amino acids

- 1) **Xanthoproteic Test:** The extracts were treated with few drops of concentrated Nitric acid. Formation of yellow colour indicates the presence of proteins.
- 2) **Ninhydrin Test:** To the extract, 0.25% w/v Ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

Detection of Diterpenes

- 1) **Copper acetate test:** Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

Test for Gum and Mucilage

To 1ml of extract add 2.5 ml of absolute alcohol and stirring constantly. Then the precipitate was dried in air and examine for its swelling properties. Swelling was observed that will indicate presence of gum and mucilage.

Test for Fixed oils and Fats

- 1) **Spot Test:** A small quantity of extract is pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oils.

Test for Quinones

Extract was treated with sodium hydroxide blue or red precipitate indicates the presence of Quinones.

HPLC - High Performance Liquid Chromatography (HPLC)^[7]

HPLC is a technique in analytical chemistry which is used to separate the components in a mixture, to identify each component, and to quantify each component. It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out of the column. In this study, the detection and quantitation were carried out using 515 HPLC pumps and 2489 UV/Visible detectors of Waters Company while the software used was Empower.

Two methods using different mobile phases were used for chromatographic separation of the research drugs – Method I (binary gradient method of Acetonitrile & 0.1% Phosphoric acid in Water) and Method II (binary gradient method of Methanol & 1:25 Acetic acid in Water). Results obtained during Method I have been discussed since better separation of compounds was observed during this analysis. The chromatographic conditions for Method I are as given below:

- Column : Symmetry C18, 5 μ m, 4.6x250 mm
- Run Time : 30 minutes
- Injection Volume : 20 μ l

- Wavelength (Dual) : 272 nm & 360 nm
- Solvent A : Acetonitrile
- Solvent B : 0.1% Phosphoric acid in water
- Flow rate : 1.0 ml/min.
- Pump Mode : Gradient
- Processing method :

Time (min)	%A (Acetonitrile)	%B (Phosphoric acid in water)
0	15	85
12	25	75
20	25	75
22	15	85
30	15	85



Figure 1: HPLC Instrument.

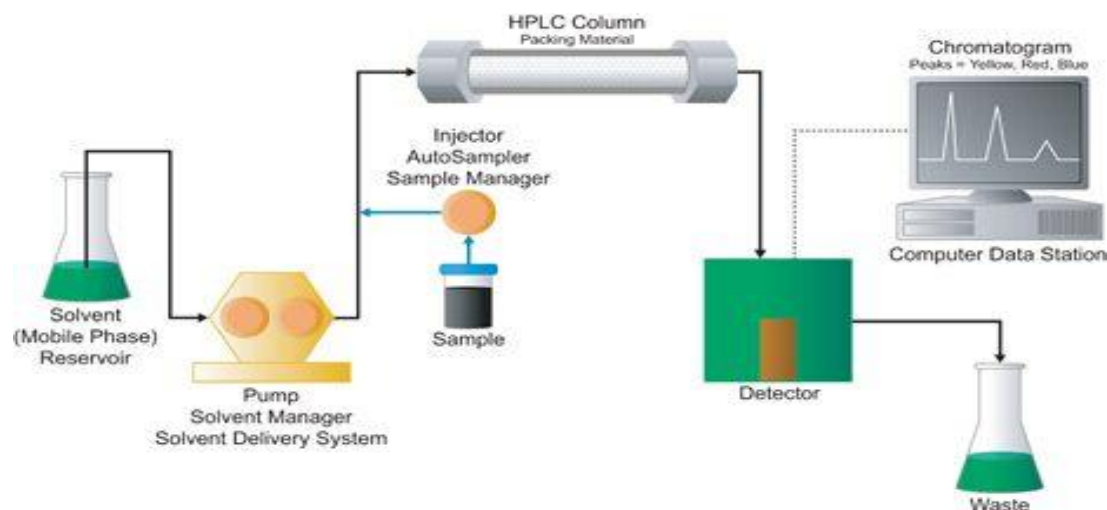


Figure 2: HPLC Mechanism.

Bio-chemical analysis^[8]

The bio-chemical analysis was done to identify the acid and basic radicals present in the sample.

Preparation of extract

5g of MPC was taken in a 250 ml clean beaker and 50 ml of distilled water was added, boiled well and allowed to cool and filtered in a 100 ml volumetric flask and made up to 100 ml with distilled water.

Preliminary Basic and Acidic radical studies

Test for basic radicals

1. Test for Potassium

To a pinch of the MPC 2 ml of sodium nitrate and 2 ml of cobalt nitrate solution in 30% glacial acetic acid was added and observed for the presence of yellow precipitate.

2. Test for Calcium

To 2 ml of the MPC extract 2 ml of 4% ammonium oxide solution was added and observed for the formation of white precipitate.

3. Test for Magnesium

To 2ml of MPC extract, drops of sodium hydroxide solution was added and watched for the appearance of white precipitate.

4. Test for Ammonium

To 2ml of MPC extract few ml of Nessler's reagent and excess of sodium hydroxide solution are added for the appearance of brown colour.

5. Test for Sodium

Hydrochloric acid was added with a pinch of the MPC sample and a paste was made and introduced into the blue flame of Bunsen burner and observed for the appearance of intense yellow colour.

6. Test for Iron (Ferrous)

The MPC extract was treated with Conc. HNO_3 and ammonium thiocyanate and waited for the appearance of blood red colour.

7. Test for Zinc

To 2 ml of the MPC extract drops of sodium hydroxide solution was added and observed for white precipitate formation.

8. Test for Aluminium

To the 2ml of the MPC extract sodium hydroxide was added in drops and noted for any characteristic changes.

9. Test for Lead

To 2 ml of MPC extract 2ml of potassium iodide solution was added and noted for yellow coloured precipitate.

10. Test for Copper

a. A pinch of MPC sample was made into a paste with concentrated HCl in a watch glass and introduced into the non-luminous part of the flame and noted for blue colour appearance.

b. To 2 ml of MPC extract excess of ammonia solution was added and observed for the appearance of blue coloured precipitate.

11. Test for Mercury

To 2ml of the MPC extract sodium hydroxide solution was added and noted for yellow precipitate formation.

12. Test for Arsenic

To 2 ml of the MPC extract 2ml of Sodium hydroxide solution was added and brown wash red precipitate if appeared was noted.

Test for acid radicals

1. Test for Sulphate

To 2 ml of the MPC extract 5% of barium chloride solution was added and observed for the appearance of white precipitate.

2. Test for Chloride

The *MPC* extract was treated with silver nitrate solution and observed for the appearance of white precipitate.

3. Test for Phosphate

The *MPC* extract was treated with ammonium molybdate and concentrated HNO_3 and observed for the appearance of yellow precipitate.

4. Test for Carbonate

The *MPC* extract was treated with concentrated HCL and observed for the appearance of effervescence.

5. Test for Fluoride & Oxalate

To 2ml of *MPC* extract 2ml of dil. acetic acid and 2ml calcium chloride solution were added and heated and watched for cloudy appearance.

6. Test for Nitrate

To 1 gm of the *MPC*, copper turnings was added and again concentrated H_2SO_4 was added, heated and the test tube was tilted vertically down and viewed for any characteristic changes.

2. RESULTS AND DISCUSSION

Organoleptic characters

Table: 2. Organoleptic characters of *Maavilingapattai Chooranam*.

Colour	Brown
Odour	Pleasant
Taste	Bitter
Texture	Fine powder
Particle size	Completely pass through sieve no 88

Physicochemical Analysis

Table: 3. Physicochemical Analysis.

S.NO	Parameter	Result
1	Ph	6.2
2	Loss on drying(at 105 ⁰ C)	5.66
3	Total ash value (%)	7.21
3	Acid Insoluble ash (%)	1.37
4	Water soluble ash (%)	3.03
5	Water soluble extraction (%)	10.54
6	Alcohol soluble extraction (%)	2.42
7	Solubility	Positive

Discussion on Physicochemical analysis

1. pH

pH is a measure of hydrogen ion concentration whether it is acidic or alkaline nature. 7.0 is neutral, above 7.0 is alkaline and below 7.0 is acidic.

The pH of the drug *Maavilingapattai Chooranam* is 6.2 which is weak acidic in nature. Acidic drug is essential for its bioavailability and effectiveness. Acidic drugs are better absorbed in stomach.^[9]

2. Moisture (Loss on drying)

The total of volatile content and moisture present in the drug was established in loss on drying. Moisture content of the drug reveals the stability and its shelf-life. High moisture content can adversely affect the active ingredient of the drug. Thus low moisture content could get maximum stability and better shelf life. Loss on drying of *Maavilingapattai Chooranam* is 5.66.^[10]

3. Total Ash

Ash constitutes are the inorganic residues obtained after complete combustion of a drug. Thus Ash value is a validity parameter to describe and to assess the degree of purity of a given drug. Total ash value of plant material indicated the amount of minerals and earthy materials present in the drug. The total ash value of *Maavilingapattai Chooranam* is 7.21 which determine the absence of inorganic content.

5. Acid insoluble ash

The acid insoluble ash value of the drug denotes the amount of siliceous matter present in the plant. The quality of the drug is better if the acid insoluble value is low. Acid insoluble ash value of *Maavilingapattai Chooranam* is 1.37.

6. Water soluble ash

Water-soluble ash is the part of the total ash content, which is soluble in water. Decreased water soluble ash value indicates easy facilitation of diffusion and osmosis mechanism. Water soluble ash value of *Maavilingapattai Chooranam* is 3.03.

7. Solubility

Solubility is the major factor that controls the bioavailability of a drug substance. It is useful to determine the form of drug and processing of its dosage form.

The most frequent causes of low oral bioavailability are attributed to poor solubility and low permeability.^[11]

MPC is soluble in major solvents and sparingly soluble in some solvents proves that its efficiency of solubility in the stomach indirectly, increasing the bio availability.

Phytochemical analysis

The phytochemical analysis of *Maavilingapattai Chooranam* result were given below:

Table: 4. Phytochemicals screening test.

S NO	Phytochemicals	Test	Result
1.	Carbohydrates	Molisch's test	Present
		Benedict's test	Present
2.	Glycosides	Modified Borntrager's test	Present
3.	Saponins	Froth test	Present
4.	Phenols	Ferric chloride test	Present
5.	Flavanoids	Alkaline reagent test	Present
		Lead acetate test	Present
6.	Diterpenes	Copper acetate test	Present
7.	Gum & Mucilage	Extract + Alcohol	Present

Discussion on Phytochemical analysis**Carbohydrate**

Carbohydrate contains plenty of antioxidants, vitamins and fiber that are necessary for our health. Carbohydrate diet can improve the liver function in people with fatty liver diseases. It also plays an important role in storage of glucose. Carbohydrates helps in fat metabolism. It plays an important role in homeostasis. Carbohydrates help us to fight inflammation and cancer, improve our digestive system, heart and bone health.^[12]

Glycosides

In the liver, glycosides helps in the process of detoxification. Glycosides have antibacterial activity, so they protect our body from bacteria and infectious diseases. Glycosides increased the intestinal motility. So it produces laxation.^[13]

Saponins

Saponins include, supporting kuffer cells in the liver and encouraging normal detoxification. In the digestive tract, saponins produce an emulsification of fat soluble molecules. Saponins bind with bile acids and helps to eliminate them from the body, preventing cholesterol from being reabsorbed. Saponins can boost the immune system, have an antioxidant effect and may even support bone strength.^[14]

Phenols

Phenols possess rich anti-oxidant property and protect the body from oxidative stress. Phenols inhibit the LDL

cholesterol levels and also reduces cell death and it regulate glucose metabolism. Phenols increase the vasodilation of blood vessels to promote circulation. It is a Effective anti-hyperglycaemic agent.^[15]

Flavanoides

It is the most important group of polyphenolic compounds in plants. Flavanoids can exert their anti-oxidant activity by scavenging the free radicals, by chelating metal ions or by inhibiting enzymatic systems responsible for free radical generation. Flavanoids are immunomodulator. It also possesses anti-microbial activity which is confirmed by the various anti-microbial assays.^[16]

Diterpenes

Diterpene has an anti-oxidant effect. Diterpenes helps to cure hypertension. It also have tumour inhibitory properties as well as a stimulating effect on the immune system. It is used widely as a stomachic.^[17]

Gum and Mucilage

It is used as a bulk laxatives. Gum and mucilage are used for their demulcent properties for cough suppression.

High Performance Liquid Chromatography (HPLC)

HPLC analysis were done. HPLC analysis performed with *Maavilingapattai Chooranam* revealed the presence of following compounds:

Table 5: Results of HPLC analysis.

S.No	Parameters	Method	Units	Results
1	Total Polyphenol as gallic acid Equivalent	Indian Pharmacopoeia 2014	mg/100g	0.09
2	Total Flavonoids as Quercetin Equivalent	TNTH/STP/FOOD/110	mg/100g	18.15
3	Total Alkaloids	TNTH/STP/FOOD /426	mg/100g	1.93
4	Total Tannin as Tannic Acid Equivalent	AOAC 20th Edn.2012, 955.35	mg/100g	0.98

HPLC analysis reveals the presence of Polyphenols, Flavanoids, Alkaloids and Tannins.

Discussion on Hplc Analysis

Polyphenols are the member of very large family of plant derived compounds which had the anti lipidogenic effect. This is mainly due to reduced fatty acid and triglycerol synthesis, increased in fatty acid oxidation and reduction of oxidative stress and inflammation. Beneficial effects

of polyphenols in the prevention and treatment of liver steatosis have been reported. Polyphenols are biomolecules which produce hepatoprotective effects which reduce the liver fat accumulation, mainly by reducing lipogenesis and by increasing fatty acid oxidation and decrease oxidative stress and inflammation are the main factors responsible for liver damage.^[18]

Flavonoids a group of plant compounds which have the beneficial effects against Non Alcoholic Fatty Liver Disease. Flavonoids prevent Hepatosteatorosis by increasing fatty acid oxidation in liver. They can also reduce caloric intake and decrease body weight and fat deposition in visceral tissues. Flavonoids are the unique

antioxidant. It also corrects dyslipidemia and blood pressure.^[19]

Tannins and alkaloids contain antioxidant effect which produces many essential effects in protecting the body.

Bio-chemical analysis

Table: 6. Results of basic radicals study.

S.NO	Parameter	Observation	Result
1	Test for Potassium	Yellow colour precipitate	Positive
2	Test For Magnesium	White colour precipitate	Positive
3	Test for Iron (Ferrous)	Blood red colour	Positive
4	Test For Zinc	Formation of white precipitate	Positive

Table: 7. Results of acid radical study.

S.no	Parameter	Observation	Result
1	Test for Sulphate	Formation of white precipitate	Positive

Discussion On Basic and Acid Radical Study

Potassium

Potassium levels may be an indicator of impending liver problems. Potassium is absorbed through the small intestine. Severe lack of potassium can disturb the liver function and if potassium level falls below 30% to 40% causes Non Alcoholic Fatty Liver Diseases. Potassium is important for maintaining the integrity of cell membranes and functions as a vital electrolyte.^[20]

Magnesium

Magnesium is essential for liver to prevent liver diseases. It enhances immune system. Depletion of magnesium levels leads to Cirrhosis and Fatty liver syndrome. It also helps to regulate blood glucose levels and aid in the production of energy and protein.^[21]

Iron

Iron is an essential micronutrient that is a critical component of oxygen transport proteins (Haemoglobin & Myoglobin). Chronic iron deficiency results in decreased haemoglobin production and anaemia which may result in chronic liver diseases. Iron is essential for oxygen transport, energy production, other cellular growth and proliferation. Iron is an essential element for blood production and also needed for energy metabolism.^[22]

Zinc

The liver plays a central role in zinc homeostasis. Zinc is a trace mineral that is essential to the normal functioning of the immune system. Zinc is essential for many metabolic and enzymatic functions. In Liver it acts as a powerful antioxidant. Deficiency of zinc leads to malabsorption syndrome and Cirrhosis of liver.^[23]

Sulphate

Sulphate reduces the increased serum enzymes in the liver and it has the liver protection property.

4. CONCLUSION

Various analyses such as physicochemical, phytochemical, biochemical and HPLC analysis were made. From the above analysis we came to know that the presence of active ingredients is responsible for its activity. Phytochemical analysis showed the presence of Carbohydrates, Glycosides, Saponins, Phenols, Flavonoids, Diterpenes, Gum & Mucilage. Biochemical analysis showed the presence of Potassium, Magnesium, Iron, Zinc and Sulphate. Thus from these results we come to know the effectiveness of the drug is due to the presence of these constituents. HPLC analysis performed with *Maavilingapattai Chooranam* revealed the presence of Polyphenols, Flavonoids, Alkaloids and Tannins. Thus the result of the study may give valuable information for further clinical studies.

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